PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 3157	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GR 02/00045	International filing date *(day/month/year)* 22.08.2002	Priority date *(day/month/year)* 22.08.2002
International Patent Classification (IPC) or both national classification and IPC A61K47/48		
Applicant PAPAIOANNOU, Dionysios et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.07.2003	15.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Lüdemann, S Telephone No. +49 89 2399-7842

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GR 02/00045

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-23	as originally filed

Claims, Numbers

2-7	received on 03.11.2004 with letter of 03.11.2004
1	received on 23.11.2004 with letter of 23.11.2004

Drawings, Sheets

1/12-12/12	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☒ the claims, Nos.: 8-11

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

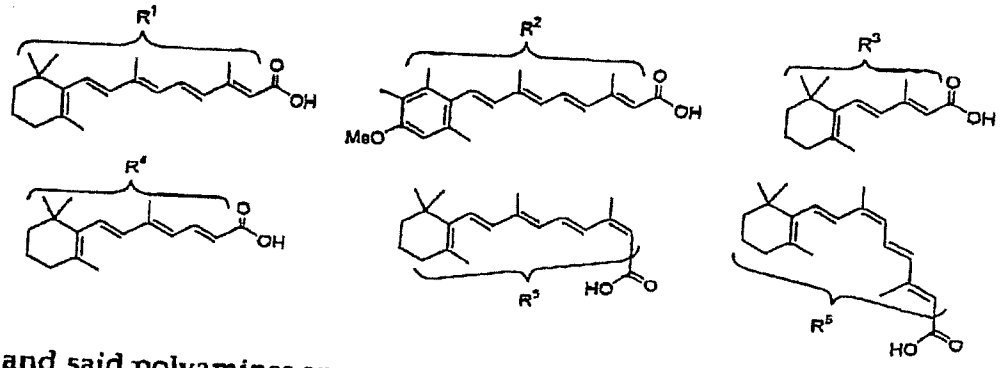
Novelty (N)	Yes:	Claims	1-7
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-7
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	-

2. Citations and explanations

   see separate sheet

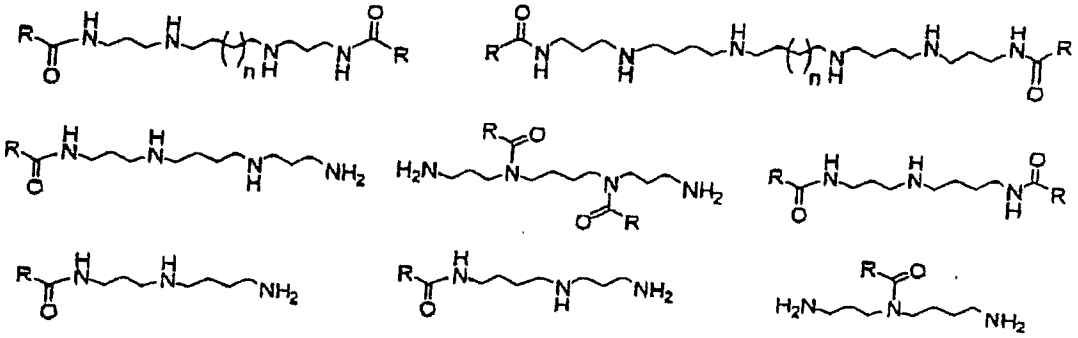
AMENDED CLAIMS

1. Conjugates of polyamines with acidic retinoids and in particular polyamine amides in which the R group of the acyl group(s) RCO is one of the retinoid residues R^1-R^6 pointed out in the following pharmaceutically important acidic retinoids and polyene chain-shortened *all-trans*-retinoic acid analogues :



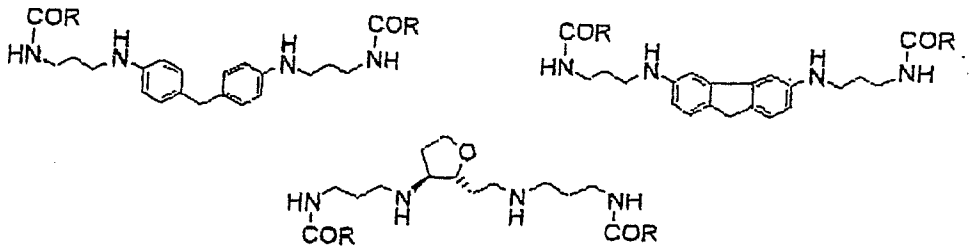
and said polyamines are :

a) Linear tri-, tetra- and hexa-amines, which conjugates have the following general formulae :

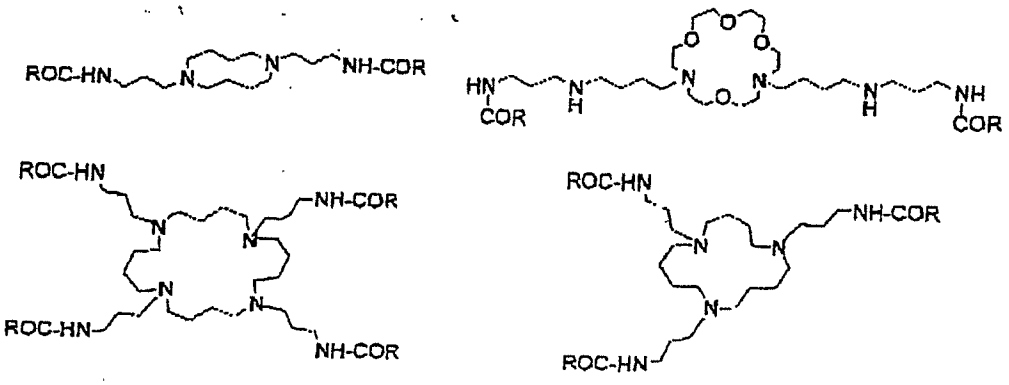


wherein n is 1 to 9

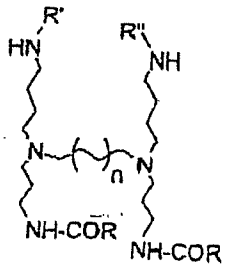
b) conformationally restricted polyamines, which conjugates have the following general formulae:



c) cyclic polyamines, which conjugates have the following general formulae :



d) branched (dimeric) polyamines, which conjugates have the following general formula :



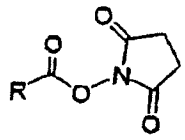
wherein

R′ is COR or $(CH_2)_3NHCOR$ and R″ is COR or $(CH_2)_3NHCOR$

and n is one of the numbers 1, 2 and 7

2. A method for the preparation of a compound according to claim 1 involving either the following two steps:

a) synthesis of compounds with the general formula



wherein R is one of the retinoid residues R^1-R^6 of claim 1, which involves esterification of acidic retinoids with HOSu in the presence of the coupling agent DCC and purification with flash column chromatography

b) direct selective acylation of the primary amino groups of polyamines with the as above obtained compounds,

or the acylation of the secondary amino groups of polyamines, protected at their primary amino functions with the trifluoroacetyl or the 9-fluorenylmethoxycarbonyl group, with the acidic retinoids of claim 1 in the presence of the coupling agent PyBrOP, followed by deprotection.

3. A method according to claim 2, which method involves the direct selective acylation of the primary amino functions of polyamines or their corresponding hydrochloride or trifluoroacetate salts with the compounds of step a) of claim 2, wherein the solvent is selected between dichloromethane, chloroform and dimethylformamide and the base, where necessary, is selected between triethylamine and diisopropylethylamine or any other tertiary amine or in general any other non-nucleophilic base.

4. A method according to claim 3 characterized in that the selective acylation of the primary amino functions of polyamines is effected with any other activated carboxylic acid derivative known to acylate selectively primary amino functions in the presence of secondary ones.

5. A method according to claim 2 characterized in that the selective mono- or bis-acylation of primary amino functions of polyamines takes place indirectly and involves the following steps :

(i) protection of the secondary amino functions of polyamines, bearing the trityl protecting group at their primary amino functions, with the 9-fluorenylmethoxycarbonyl or the trifluoroacetyl group

(ii) detritylation

(iii) mono- or bis-acylation with the compounds of step a) of claim 2

(iv) complete deprotection and purification, if necessary, by flash column chromatography.

6. A method according to claim 2 characterized in that the selective acylation of the secondary amino functions of polyamines involves the following steps:

(i) selective trifluoroacetylation of the primary amino functions of polyamines

(ii) acylation of the secondary amino functions with the acidic retinoids of claim 1 in the presence of the coupling agent PyBroP

(iii) removal of the trifluoroacetyl groups by alkaline hydrolysis.

7. Pharmaceutical preparations or products containing the compounds claimed in claim 1 for therapeutical applications in humans

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.1 Reference is made to the following documents:

D1: WO 98/34646 A (ATTERWILL CHRISTOPHER KENNETH ;PURCELL WENDY MARIA (GB); ISMAIL FY) 13 August 1998 (1998-08-13)

D2: MANFREDINI STEFANO ET AL: "Retinoic acid conjugates as potential antitumor agents: Synthesis and biological activity of conjugates with Ara-A, Ara-C, 3(2H)-furanone, and aniline mustard moieties." JOURNAL OF MEDICINAL CHEMISTRY, vol. 40, no. 23, 7 November 1997 (1997-11-07), pages 3851-3857, XP002236863 ISSN: 0022-2623

D3: US-B-6 344 2061 (GIACOMONI PAOLO ET AL) 5 February 2002 (2002-02-05)

D4: KARIGIANNIS GEORGE ET AL: "Structure, biological activity and synthesis of polyamine analogues and conjugates." EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, 2000, pages 1841-1863, XP002236864

D5: PAPADIMOU EVANGELIA ET AL: "Inhibition of ribonuclease P activity by retinoids." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 38, pages 24375-24378, XP002237316 ISSN: 0021-9258

1.2 D1 (WO9834646), which is considered as the closest prior art, discloses antioxidants, e.g. carotene-like substances like retinoic acid linked to targeting moiety such as polyamines, like spermine and spermidine for the treatment of neurodegenerative disorders. Conjugates of polyamines with **all-trans**-retinoic acids analogues with the structures as disclosed in present claim 1 are not disclosed.

1.3 D2 (XP002236863) discloses diamine linked to retinoid disclosed for the treatment of tumors. Substances according to claim 1 are not disclosed.

1.4 In D3 (US6344206B1), composition comprising retinol and a polyamine polymer are disclosed. Substances according to claim 1 are not disclosed.

1.5 D4 (XP002236864) is a review dealing with polyamine analogues and conjugates.

Substances according to claim 1 are not disclosed.

1.6 D5 (XP002237316) discloses the inhibition of ribonuclease P activity by retinoids. Substances according to claim 1 are not disclosed.

1.7 None of the documents D1-D5 discloses the **all-trans**-retinoic acids analogues with the structures as disclosed in present claim 1.

1.8 Furthermore, D1 does not provide any example of how to prepare conjugates of retinoic acids with spermine or spermidine. The examples provided describe synthesis of conjugates via a one-pot reaction of a benzopyran-type antioxidant with a benzylic-type bromine atom used to alkylate the alpha-amino function of an α,ω-diaminoalkane. The present method differs from D1 in that the conjugates are obtained by succinimidyl esters of all-trans-retinoic acids and consecutive purification by flash column chromatography. This is not disclosed or suggested by any of the documents D1-D5.

1.9 Therefore, claims 1-7 fulfill the requirements of Art. 33(2) and 33(3) PCT.